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Cortical Photostimulation Technology for Vision Prosthesis

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CONTRACTING ORGANIZATION:

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The overall goal of the project is to evaluate the feasibility of using photochemical stimulation of cortical neurons as the basis for a visual prosthesis. The most significant result from this reporting period is the successful synthesis of a "caged" glutamate. Caged glutamates are biological inert molecules that, upon exposure to a focused flash of light, are rapidly transformed into the bioactive neurotransmitter, glutamate, which can directly stimulate cortical neurons. The new caged molecule has an absorption peak in the visible wavelength range, at 458 nm — this exceeds the original expectation of absorption peaking at ~410 nm.

15. SUBJECT TERMS
Visual prosthesis, photostimulation, photorelease, uncaging, caged glutamate

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1. Introduction

The goal of this project is to develop a photostimulation technology that can serve as the basis of a new visual prosthesis for wounded warriors who have lost their anterior visual system (i.e., the eyes and/or optic nerves). At the core of the technology is a "caged glutamate", a molecule that is inert (or "caged") until it is transformed by a focused light flash into the active neurotransmitter molecule, glutamate, which then can stimulate neurons in the visual cortex to evoke visual perception. The photochemical process that transforms the caged glutamate into active glutamate is referred to as "uncaging" or "photorelease". The project comprises three key aims: 1) development of a caged glutamate that absorbs light in the visible wavelength range, 2) testing of the new caged glutamate in vitro and in vivo, 3) validation of the photostimulation approach in the mouse cortex.

2. Keywords

Visual prosthesis, photostimulation, photorelease, uncaging, caged glutamate

3. Accomplishments

► Major goals of the project (year 1)

Major goals/task for the 1st year of the project, as outlined in the approved SOW are summarized in the table below.

Specific Aim 1: Develop a photoreleasable neurotransmitter optimized for in vivo photostimulation of the cortex.	**Proposed month of completion**	**Actual completion month**
Major Task 1: Animal protocols		
●***Milestone*** *ACURO approval obtained*	4	12
Major Task 2: Synthesis and characterization of caged glutamate, oxNI-Glu		
●***Milestone*** *Synthesis of caged Glu completed*	5	8*, 12
●***Milestone*** *Characterization of caged Glu*	7	ongoing (50%)
Major Task 3. Scale-up production of caged Glu for in vivo animal studies.		
●***Milestone*** *Scaled up caged Glu synthesis completed*	10	ongoing (15%)
●***Milestone*** *HPLC acquisition and set up, and purification ongoing*	12	ongoing (50%)
Major Task 4: Test in vivo toxicity of new compound		
●***Milestone*** *Brain tissue harvest completed*	12	ongoing (15%)

*When obstacles arose during the synthesis of oxNI-Glu, we prepared an alternative caged glutamate, cmoNI-Glu. In case the originally proposed oxNI-Glu could not be synthesized, cmoNI-Glu would be a serviceable alternative; synthesis of cmoNI-Glu was completed in month 8. Synthesis of oxNI-Glu ultimately proved tractable, however.

► Accomplishments under the major goals/tasks

The most important accomplishments are two, one administrative and one scientific. The administrative accomplishment was obtaining approvals for the animal use protocols from the IACUCs of the two participating university campuses, and from the USAMRMC ACURO. The scientific accomplishment relate to the synthesis of the caged glutamate, as summarized below.

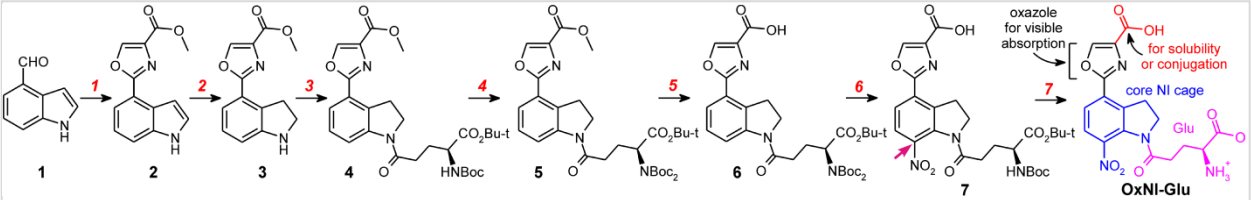

Fig. 1. Synthesis of oxNI-Glu (originally proposed). *Reagents and conditions*: ***1.*** *i.* Serine-OMe·HCl, K_2CO_3, DMA, *ii*. $BrCCl_3$, DBU; ***2.*** $NaCNBH_3$, HOAc; ***3.*** *N*-Boc-*L*-Glu-O(*tert*-Bu), EDC·HCl, MeCN; ***4.*** di-*tert*-butyl dicarbonate, Et_3N, DMAP, CH_2Cl_2, reflux; ***5.*** *i.* NaOH, MeOH, *ii.* citric acid; ***6.*** *i.* Claycop, acetic anhydride/CCl_4, *ii.* 1 *M* TFA; ***7.*** TFA. Abbreviations: Ac = CH_3CO; *t*-Bu = *tert*-butyl; Boc = *tert*-butoxycarbonyl; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DMA = *N,N*-dimethylacetamide; DMAP = 4-dimethylaminopyridine; EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Et = CH_2CH_3; Me = CH_3; TFA = trifluoroacetic acid.

The originally proposed synthesis of oxNI-Glu shown in Figure 1. In practice, reaction step 6 failed—Claycop ($Cu(NO_3)_2$ on Montmorillonite clay) in the presence of acetic anhydride failed to nitrate compound **6** in ring position 7 (indicated by magenta arrowhead in compound **7**).

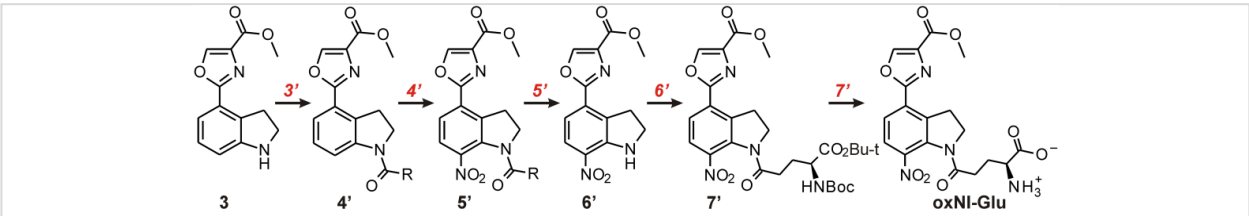

Fig. 2. Revised synthesis of oxNI-Glu. *Reagents and conditions*: ***3'.*** R=CH_3: acetic anhydride, Et_3N; R=H: pivalic formic anhydride, Et_3N; ***4'.*** $Bi(NO_3)_3$, acetic anhydride, CH_2Cl_2; ***5'.*** NaOMe, MeOH; ***6'.*** *N*-Boc-*L*-Glu(Cl)-O(*tert*-Bu), Et_3N, DMAP, CH_2Cl_2; ***7'.*** *i.* NaOH, *ii*. TFA. Abbreviations same as in Fig. 1.

Therefore, we investigated alternative nitration reactions and catalysts. These included substitution in position 7 with bis(trifluoroacetoxy)thallium, followed by displacement with $NaNO_2$, as well as catalytic nitration with $Cu(NO_3)_2$, $Fe(NO_3)_3$, and $Bi(NO_3)_3$, and Claycop under various conditions. The best reaction yields were achieved with $Bi(NO_3)_3$. This finding, along with other chemical considerations, led us to redesign the oxNI-Glu synthesis as shown in Figure 2 (since the first 3 compounds in the synthesis sequence are identical to those in Figure 1, the sequence in Figure 2 is abbreviated and begins with compound **3**). The key intermediate **5'** (R=CH_3) formed crystals of very good quality, and X-ray crystallography confirmed the desired structure with the nitro (NO_2) group in ring position 7 (Figure 3).

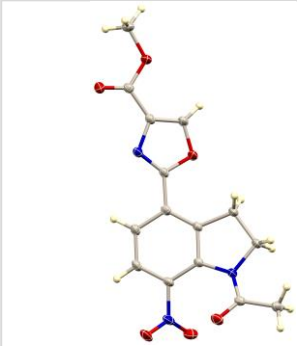

Fig. 3. X-ray crystallographic structure of compound 5' (in Fig. 2) Atoms: C: gray; O: red; N: blue; H: ivory.

As noted in the "major goals" section above, as we were exploring the synthesis of oxNI-Glu, we synthesized an alternative caged glutamate, cmoNI-Glu, in case we could not synthesize oxNI-Glu. The synthesis of cmoNI-Glu is outlined in Figure 4 (this was also provided in the 3rd quarterly report). In the synthesis, deprotection of *tert*-butyl groups was performed in

two separate steps (***8*** and ***9***) with two different concentrations of trifluoroacetic acid, because part of the penultimate product (product of reaction ***8***) was saved for potential conjugation to hydrophilic polymers.

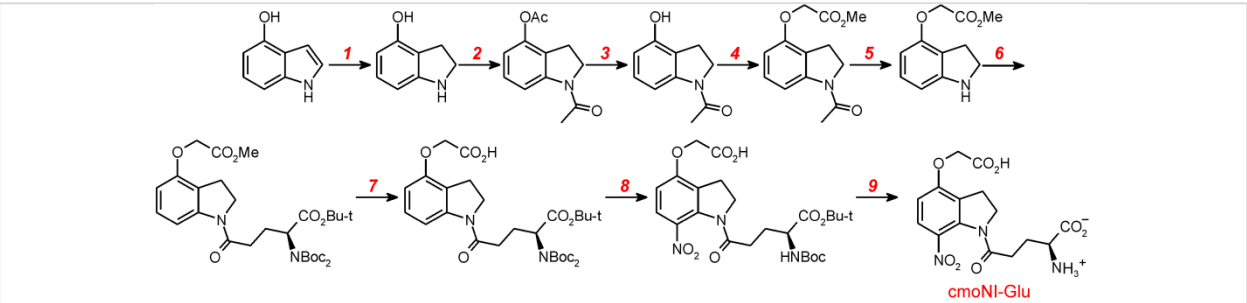

Fig. 4. Synthesis of oxNI-Glu. *Reagents and conditions*: ***1***. $NaCNBH_3$, HOAc; ***2***. Acetic anhydride; ***3***. *i.* NaOH; *ii.* HCl; ***4***. K_2CO_3, $BrCH_2CO_2CH_3$, acetone; ***5***. HCl, H_2O/MeOH, reflux; ***6***. *i. N*-Boc-*L*-Glu-O(*tert*-Bu), EDC·HCl, MeCN; *ii.* di-*tert*-butyl dicarbonate, Et_3N, DMAP, CH_2Cl_2, reflux; ***7***. NaOH, H_2O/MeOH ***8***. *i.* Claycop, acetic anhydride, CCl_4; *ii.* 1 *M* TFA; ***9***. TFA. Abbreviations same as in Fig. 1.

An important and gratifying feature of the new oxNI cage is its UV-visible absorption spectrum, which is shown in Figure 5. There is a prominent absorption peak in the blue region of the visible spectrum. The peak wavelength of λ_{max} = 458 nm is much more red-shifted than we had initially anticipated (we estimated $\lambda_{max} \approx$ 410 nm in the original proposal). This validates our approach of using the oxazole moiety to red-shift the absorption spectrum of the cage (see markings on the oxNI-Glu structure in Figure 1). Furthermore, the large red-shift enables the use of longer-wavelength solid-state lasers for photostimulation, which permits better penetration into brain tissue.

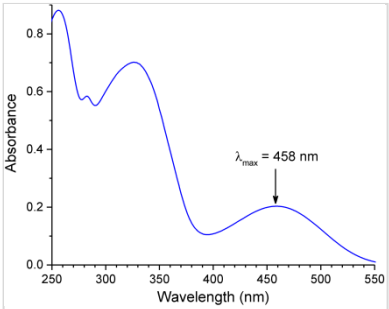

Fig. 5. UV-visible spectrum of oxNI cage

Characterization of the properties of oxNI-Glu, most important being photolysis kinetics and quantum yield, are under way. Scale-up of the synthesis is also ongoing.

With the approval of the animal protocols, toxicological tests of oxNI-Glu are being conducted in mice.

An automated operant conditioning cage has been designed and built for training mice to display a defined behavior in response to a specific sound stimulus. This device is for use in the second year of the project (see item 5 under “Planned activities in the next reporting period” below).

► Opportunities for training and professional development

Nothing to report.

► Dissemination of results to communities of interest

Nothing to report.

► Planned activities in the next reporting period

To achieve the goals of the project, the following key tasks will be performed in the next reporting period.

1. Complete scale-up synthesis and purification of oxNI-Glu.
2. Complete toxicological testing of oxNI-Glu in mice.

3. Validate photostimulation with oxNI-Glu in mouse cortical slices in vitro by establishing the proper photostimulation parameters (optical power, pulse duration, maximum stimulation frequency) and defining the spatial resolution of the technique.
4. Validate photostimulation with oxNI-Glu in vivo by determining the maximum optical power that can be tolerated (the biological damage threshold), and the achievable spatial resolution.
5. Functionally validate photostimulation: Use automated operant conditioning cages* to train mice to respond to a specific tone by displaying a particular behavior (licking of water spout), then use photostimulation to activate the region of the cortical tonotopic map that corresponds to the specific tone to evoke the conditioned behavior.

*The automated conditioning cages have been designed and built. They are needed for this project and for another, separately funded project (see entry for Dr. Nikolas Francis, under Section 7, “Participants and other collaborating institutions”).

4. Impact

► Impact on the development of the principal discipline(s) of the project

Development of oxNI cage is a significant advance: it demonstrates 1) that, by rational design, light absorption by cages of the NI class can be red-shifted into the visible wavelength range, and 2) that such red-shifting makes a visual prosthesis based on caged glutamate photochemistry more feasible.

► Impact on other disciplines

Nothing to report.

► Impact on technology transfer

Nothing to report.

► Impact on society beyond science and technology

Nothing to report.

5. Changes/Problems

► Changes in approach and reasons for change

Nothing to report.

► Actual or anticipated problems or delays and actions or plans to resolve them

As summarized in Section 3, there were some delays in meeting milestones. Several circumstances contributed to the delays. First, although the award began with the official award notice on 4/15/2016, account set-up and funds transfer took almost two months to complete. Second, new animal protocols required for the project took longer to gain the proper approvals, but are now fully approved, allowing animal work to proceed. Third, the chemical syntheses proved more challenging than anticipated — this is typical of synthetic organic chemistry, where reactions that work well in one compound work poorly in other, similar compounds. By redoubling our efforts, we have minimized the delays, and expect to be back on schedule within the next quarter.

► Changes that had a significant impact on expenditures

Nothing to report.

► Significant changes in use/care of vertebrate animals, biohazards, and/or select agents

Nothing to report.

6. Products

Nothing to report.

7. Participants & other collaborating institutions

► Individuals who worked on the project

Name	Joseph P. Y. Kao
Project Role	P.I.
Researcher identifier	eRA Commons: KAOJOESPH
Person months worked	2
Contribution	Overseeing and coordinating project, synthesis and characterization of caged glutamates
Funding support	This award (W81XWH-16-2-0011)

Name	Patrick O. Kanold
Project Role	Site P.I. at Univ. of Maryland, College Park
Researcher identifier	eRA Commons: Pkanold
Person months worked	1
Contribution	Design of automated operant conditioning cage, set up of in vitro photostimulation instrumentation
Funding support	This award (W81XWH-16-2-0011)

Name	Nathaniel D. A. Dirda
Project Role	Chemical technician
Researcher identifier	
Person months worked	10
Contribution	Performed organic synthesis, purification, and routine characterization of caged glutamates under the direction of Dr. Kao.
Funding support	This award (W81XWH-16-2-0011)

Name	Eric A. Legenzov
Project Role	Graduate student

Researcher identifier	eRA Commons: ELEGENZOV
Person months worked	1
Contribution	Setting up rig for in vivo toxicology and perfusion/fixation.
Funding support	This award (W81XWH-16-2-0011)

Name	Nikolas A. Francis
Project Role	Post-doctoral fellow
Researcher identifier	eRA Commons: nikolasfrancis
Person months worked	1
Contribution	Construction and testing of automated operant conditioning cage
Funding support	U01NS90569 (NIH/NINDS, BRAIN Initiative)

► Change in the active other support of the PD/PI(s) or senior/key personnel

The PI, Dr. Joseph Kao (University of Maryland, Baltimore), and Dr. Kanold (site PI at University of Maryland, College Park), jointly received a 1-year Phase 1 research grant from the Medical Technology Enterprise Consortium (MTEC):

Award Number: MTEC 2017-607-001 (Phase 1)
Title: Photochemical Brain Machine Interface: Platform for Vision Prosthesis
Period: 02/20/2017 – 02/19/2018

This additional funding does not impact effort levels in the present project.

► Other organizations that were involved as partners

Nothing to report.

8. Special reporting requirements

None.

9. Appendices

None.